

OIPE

RAW SEQUENCE LISTING DATE: 09/07/2001 PATENT APPLICATION: US/09/509,945 TIME: 10:31:25

```
1 <110> APPLICANT: HAMADA, Kazuyuki et al.
      2 <120> TITLE OF INVENTION: MUTANT BARNASE GENE AND TRANSGENIC PLANT TRANSFORMED BY SAID
GENE
      3 <130> FILE REFERENCE: 0230-0148P
      4 <140> CURRENT APPLICATION NUMBER: US/09/509,945
C--> 5 <141> CURRENT FILING DATE: 2000-04-04
                                                         ENTERED
      6 <160> NUMBER OF SEQ ID NOS: 7
      7 <170> SOFTWARE: PatentIn version 3.1
     9 <210> SEQ ID NO: 1
     10 <211> LENGTH: 343
     11 <212> TYPE: DNA
     12 <213> ORGANISM: Bacillus amyloliquefaciens
     13 <220> FEATURE:
     14 <221> NAME/KEY: misc_feature
     15 <223> OTHER INFORMATION: wild type barnase gene
     16 <221> NAME/KEY: CDS
     17 <222> LOCATION: (1)..(336)
     18 <223> OTHER INFORMATION:
     19 <400> SEQUENCE: 1
     2.0
              atg gta ccg gtt atc aac acg ttt gac ggg gtt gcg gat tat ctt cag
                                                                                     48
     21
              Met Val Pro Val Ile Asn Thr Phe Asp Gly Val Ala Asp Tyr Leu Gln
     22
                                                  10
     23
                                                                                     96
              aca tat cat aag cta cct gat aat tac att aca aaa tca gaa gca caa
     24
              Thr Tyr His Lys Leu Pro Asp Asn Tyr Ile Thr Lys Ser Glu Ala Gln
     25
                          20
                                              25
     26
              gcc ctc ggc tgg gtg gca tca aaa ggg aac ctt gca gac gtc gct ccg
                                                                                    144
     27
              Ala Leu Gly Trp Val Ala Ser Lys Gly Asn Leu Ala Asp Val Ala Pro
     28
                                          40
     29
              ggg aaa agc atc ggc gga gac atc ttc tca aac agg gaa ggc aaa ctc
                                                                                    192
     30
              Gly Lys Ser Ile Gly Gly Asp Ile Phe Ser Asn Arg Glu Gly Lys Leu
     31
                                      55
     32
              ccg ggc aaa agc gga cga aca tgg cgt gaa gcg gat att aac tat aca
                                                                                    240
     33
              Pro Gly Lys Ser Gly Arg Thr Trp Arg Glu Ala Asp Ile Asn Tyr Thr
     34
                                  70
                                                      75
     35
                                                                                    288
              tca ggc ttc aga aat tca gac cgg att ctt tac tca agc gac tgg ctg
     36
             Ser Gly Phe Arg Asn Ser Asp Arg Ile Leu Tyr Ser Ser Asp Trp Leu
     37
     38
              att tac aaa aca acg gac cat tat cag acc ttt aca aaa atc aga taa
                                                                                    336
     39
              Ile Tyr Lys Thr Thr Asp His Tyr Gln Thr Phe Thr Lys Ile Arg
     40
             ggtaacc
                                                                                    343
    43 <210> SEQ ID NO: 2
    44 <211> LENGTH: 111
    45 <212> TYPE: PRT
    46 <213> ORGANISM: Bacillus amyloliquefaciens
    47 <220> FEATURE:
    48 <221> NAME/KEY: misc_feature
    49 <223> OTHER INFORMATION: wild type barnase gene
```

```
50 <400> SEQUENCE: 2
51
         Met Val Pro Val Ile Asn Thr Phe Asp Gly Val Ala Asp Tyr Leu Gln
52
53
         Thr Tyr His Lys Leu Pro Asp Asn Tyr Ile Thr Lys Ser Glu Ala Gln
54
                     20
                                          25
55
         Ala Leu Gly Trp Val Ala Ser Lys Gly Asn Leu Ala Asp Val Ala Pro
56
                                      40
                                                          45
57
         Gly Lys Ser Ile Gly Gly Asp Ile Phe Ser Asn Arg Glu Gly Lys Leu
58
             50
                                  55
59
         Pro Gly Lys Ser Gly Arg Thr Trp Arg Glu Ala Asp Ile Asn Tyr Thr
60
                              70
                                                  75
61
         Ser Gly Phe Arg Asn Ser Asp Arg Ile Leu Tyr Ser Ser Asp Trp Leu
62
                                              90
         Ile Tyr Lys Thr Thr Asp His Tyr Gln Thr Phe Thr Lys Ile Arg
63
64
66 <210> SEQ ID NO: 3
67 <211> LENGTH: 342
68 <212> TYPE: DNA
69 <213> ORGANISM: Artificial Sequence
70 <220> FEATURE:
71 <223> OTHER INFORMATION: A mutant barnase gene derived from Bacillus amyloliquefaciens
72 <400> SEQUENCE: 3
73
         atggtaccgg ttattcaaca cgtttgacgg ggttgcggat tatcttcaga catatcataa
                                                                                 60
74
         gctacctgat aattacatta caaaatcaga agcacaagcc ctcggctggg tggcatcaaa
                                                                                120
75
         agggaacctt gcagacgtcg ctccggggaa aagcatcggc ggagacatct tctcaaacag
                                                                                180
76
         ggaaggcaaa ctcccgggca aaagcggacg aacatggcgt gaagcggata ttaactatac
                                                                                240
77
         atcaggette agaaatteag aceggattet ttactcaage gactggetga tttacaaaac
                                                                                300
         aacggaccat tatcagacct ttacaaaaat cagtaatcta ga
                                                                                342
80 <210> SEQ ID NO: 4
81 <211> LENGTH: 6548
82 <212> TYPE: DNA
83 <213> ORGANISM: Escherichia coli LE392
84 <220> FEATURE:
85 <221> NAME/KEY: misc_feature
86 <223> OTHER INFORMATION: Clone: pTS172
87 <400> SEQUENCE: 4
88
         aattcaagct tgacgtcagg tggcactttt cggggaaatg tgcgcggaac ccctatttgt
                                                                                 60
89
         ttatttttct aaatacattc aaatatgtat ccgctcatga gacaataacc ctgataaatg
                                                                                120
         cttcaataat attgaaaaag gaagagtatg agtattcaac atttccgtgt cgcccttatt
90
                                                                                180
91
         cccttttttg cggcattttg ccttcctgtt tttgctcacc cagaaacgct ggtgaaagta
                                                                                240
92
         aaagatgctg aagatcagtt gggtgcacga gtgggttaca tcgaactgga tctcaacagc
                                                                                300
93
         ggtaagatcc ttgagagttt tcgccccgaa gaacgttttc caatgatgag cacttttaaa
                                                                                360
94
         gttctgctat gtggcgcggt attatcccgt attgacgccg ggcaagagca actcggtcgc
                                                                                420
95
         cgcatacact attctcagaa tgacttggtt gagtactcac cagtcacaga aaagcatctt
                                                                                480
96
         acggatggca tgacagtaag agaattatgc agtgctgcca taaccatgag tgataacact
                                                                                540
97
         gcggccaact tacttetgac aacgategga ggaccgaagg agetaaeege ttttttgcae
                                                                                600
98
         aacatggggg atcatgtaac tcgccttgat cgttgggaac cggagctgaa tgaagccata
                                                                                660
99
         ccaaacgacg agcgtgacac cacgatgcct gtagcaatgg caacaacgtt gcgcaaacta
                                                                                720
100
         ttaactggcg aactacttac tctagcttcc cggcaacaat taatagactg gatggaggcg
                                                                                 780
```

101		caggaccact					840
102		ccggtgagcg	-				900
103		gtatcgtagt	_			•	960
104	aatagacaga	tcgctgagat	aggtgcctca	ctgattaagc	attggtaact	gtcagaccaa	1020
105	gtttactcat	atatacttta	gattgattta	aaacttcatt	tttaatttaa	aaggatctag	1080
106		tttttggctc					1140
107	cactgagcgt	cagaccccgt	agaaaagatc	aaaggatctt	cttgagatcc	tttttttctg	1200
108	cgcgtaatct	gctgcttgca	aacaaaaaa	ccaccgctac	cagcggtggt	ttgtttgccg	1260
109	gatcaagagc	taccaactct	ttttccgaag	gtaactggct	tcagcagagc	gcagatacca	1320
110		ttctagtgta					1380
111	cctacatacc	tcgctctgct	aatcctgtta	ccagtggctg	ctgccagtgg	cgataagtcg	1440
112	tgtcttaccg	ggttggactc	aagacgatag	ttaccggata	aggcgcagcg	gtcgggctga	1500
113	acggggggtt	cgtgcacaca	gcccagcttg	gagcgaacga	cctacaccga	actgagatac	1560
114	ctacagcgtg	agcattgaga	aagcgccacg	cttcccgaag	ggagaaaggc	ggacaggtat	1620
115	ccggtaagcg	gcagggtcgg	aacaggagag	cgcacgaggg	agcttccagg	gggaaacgcc	1680
116	tggtatcttt	atagtcctgt	cgggtttcgc	cacctctgac	ttgagcgtcg	atttttgtga	1740
117	tgctcgtcag	gggggcggag	cctatggaaa	aacgccagca	acgcggcctt	tttacggttc	1800
118	ctggcctttt	gctggccttt	tgctcacatg	ttctttcctg	cgttatcccc	tgattctgtg	1860
119	gataaccgta	ttaccgcctt	tgagtgagct	gataccgctc	gccgcagccg	aacgaccgag	1920
120		cagtgagcga					1980
121	gcgcgttggc	ctgatcagaa	ttcatatgca	cgtgttcccg	atctagtaac	atagatgaca	2040
122	ccgcgcgcga	taatttatcc	tagtttgcgc	gctatatttt	gttttctatc	gcgtattaaa	2100
123	tgtataattg	cgggactcta	atcataaaaa	cccatctcat	aaataacgtc	atgcattaca	2160
124	tgttaattat	tacatgctta	acgtaattca	acagaaatta	tatgataatc	atcgcaagac	2220
125	cggcaacagg	attcaatctt	aagaaacttt	attgccaaat	gtttgaacga	tctgcttcgg	2280
126	aggttacctt	atctgatttt	tgtaaaggtc	tgataatggt	ccgttgtttt	gtaaatcagc	2340
127	cagtcgcttg	agtaaagaat	ccggtctgaa	tttctgaagc	ctgatgtata	gttaatatcc	2400
128	gcttcacgcc	atgttcgtcc	gcttttgccc	gggagtttgc	cttccctgtt	tgagaagatg	2460
129	tctccgccga	tgcttttccc	cggagcgacg	tctgcaaggt	tcccttttga	tgccacccag	2520
130	ccgagggctt	gtgcttctga	ttttgtaatg	taattatcag	gtagcttatg	atatgtctga	2580
131	agataatccg	caaccccgtc	aaacgtgttg	ataaccggta	ccatcgcgac	ggcttgatgg	2640
132	atctcttgct	ggacaccggg	atgctaggat	gggttatcgt	ggccggcgtg	cgtgtgtggc	2700
133	ttttgtaggc	gccggcgacg	gcgggggcaa	tgtggcaggt	gagtcacggt	gcaagcgtgc	2760
134	gcaagtgact	gcaacaacca	aggacggtca	tggcgaaagc	acctcacgcg	tccaccgtct	2820
135	acaggatgta	gcagtagcac	ggtgaaagaa	gtgttgtccc	gtccattagg	tgcattctca	2880
136	ccgttggcca	gaacaggacc	gttcaacagt	taggttgagt	gtaggacttt	tacgtggtta	2940
137	atgtatggca	aatagtagta	aattttgccc	ccattggtct	ggctgagata	gaacatattc	3000
138	tggaaagcct	ctagcatatc	ttttttgaca	gctaaacttt	gcttcttgcc	ttcttggtct	3060
139	agcaatgacg	ttgcccatgt	cgtggcaaac	atctggtaag	gtaactgtat	tcgtttgttc	3120
140	ccttcaacgg	ctcaatcccc	acaggccaag	ctatcctttc	cttggcagta	taggctcctt	3180
141	gagagattat	actaccattt	ttaagtgctt	ataaagacga	tgctctctaa	ccagatcgat	3240
142	cagaaacaca	aagttttagc	agcgtaatat	cccacacaca	tacacacacg	aagctatgcc	3300
143	tcctcatttt	ccgagagatt	ctgacagtga	ccagaatgtc	agaatgccat	ttcatgggca	3360
144	caagtcgatc	cacaagcttc	ttggtggagg	tcaaggtgtg	${\tt ctattattat}$	tcgctttcta	3420
145	ggaaattatt	cagaattagt	gccttttatc	ataacttctc	tctgagccga	tgtggttttg	3480
146	gatttcattg	ttgggagcta	tgcagttgcg	gatattctgc	tgtggaagaa	caggaactta	3540
147	tctgcggggg	tccttgctgg	ggcaacattg	atatggttcc	tgttcgatgt	agtagaatac	3600
148		cgctcctttg					3660
149	tcaaatgccg	caccactctt	ggacaggtat	tagctttatt	tcctgtggag	atggtagaaa	3720

```
150
                                                                                3780
          actcagctta cagaaatggc atttcacgta gtataacgca agacattagg tactaaaact
151
          caactaactg tttccgaatt tcagggcccc tccaaggatc ccagaaatca tcatctctga
                                                                                3840
152
          acatgccttc agagaaatgg cattgaccgt ccattacaaa ctaacgtaca ctgtatctgt
                                                                                3900
153
          tctttacgac attgcatgtg gaaaggatct gaagagattt ctcctggtac ataataatct
                                                                                3960
154
          actcctttgc tacgttaata agagatgtaa aaacatgcaa cagttccagt gccaacattg
                                                                                4020
155
          tccaaggatt gtgcaattct ttctggagcg ctaaaattga ccagattaga cgcatcagaa
                                                                                4080
156
          tattgaattg cagagttagc caataatcct cataatgtta atgtgctatt gttgttcact
                                                                                4140
157
          actcaatata gttctggact aacaatcaga ttgtttatga tattaaggtg gttggatctc
                                                                                4200
158
          tattggtatt gtcggcgatt ggaagttctt gcagcttgac aagtctacta tatattggta
                                                                                4260
159
          ggtattccag ataaatatta aattttaata aaacaatcac acagaaggat ctgcggccgc
                                                                                4320
160
          tagcctaggc ccgggcccac aaaaatctga gcttaacagc acagttgctc ctctcagagc
                                                                                4380
161
          agaatcgggt attcaacacc ctcatatcaa ctactacgtt gtgtataacg gtccacatgc
                                                                                4440
162
                                                                                4500
          cggtatatac gatgactggg gttgtacaaa ggcggcaaca aacggcgttc ccggagttgc
163
          acacaagaaa tttgccacta ttacagaggc aagagcagca gctgacgcgt acacaacaag
                                                                                4560
164
          tcagcaaaca gacaggttga acttcatccc caaaggagaa gctcaactca agcccaagag
                                                                                4620
165
          ctttgctaag gccctaacaa gcccaccaaa gcaaaaagcc cactggctca cgctaggaac
                                                                                4680
166
                                                                                4740
          caaaaaggccc agcagtgatc cagccccaaa agagatctcc tttgccccgg agattacaat
167
                                                                                4800
          ggacgatttc ctctatcttt acgatctagg aaggaagttc gaaggtgaag gtgacgacac
168
          tatgttcacc actgataatg agaaggttag cctcttcaat ttcagaaaga atgctgaccc
                                                                                4860
169
          acagatggtt agagaggcct acgcagcagg tctcatcaag acgatctacc cgagtaacaa
                                                                                4920
170
          tctccaggag atcaaatacc ttcccaagaa ggttaaagat gcagtcaaaa gattcaggac
                                                                                4980
171
          taattgcatc aagaacacag agaaagacat atttctcaag atcagaagta ctattccagt
                                                                                5040
172
          atggacgatt caaggcttgc ttcataaacc aaggcaagta atagagattg gagtctctaa
                                                                                5100
173
          aaaggtagtt cctactgaat ctaaggccat gcatggagtc taagattcaa atcgaggatc
                                                                                5160
174
          taacagaact cgccgtgaag actggcgaac agttcataca gagtctttta cgactcaatg
                                                                                5220
175
          acaagaagaa aatcttcgtc aacatggtgg agcacgacac tctggtctac tccaaaaatg
                                                                                5280
176
          tcaaagatac agtctcagaa gaccaaaggg ctattgagac ttttcaacaa aggataattt
                                                                                5340
177
          cgggaaacct cctcggattc cattgcccag ctatctgtca cttcatcgaa aggacagtag
                                                                                5400
178
                                                                                5460
          aaaaggaagg tggctcctac aaatgccatc attgcgataa aggaaaggct atcattcaag
179
          atgcctctgc cgacagtggt cccaaagatg gacccccacc cacgaggagc atcgtggaaa
                                                                                5520
180
          aagaagacgt tccaaccacg tcttcaaagc aagtggattg atgtgacatc tccactgacg
                                                                                5580
181
                                                                                5640
          taagggatga cgcacaatcc cactatcctt cgcaagaccc ttcctctata taaggaagtt
182
                                                                                5700
          catttcattt ggagaggaca cgctgaaatc accagtctct ctctataaat ctatctctct
183
                                                                                5760
          ctctataacc atggacccag aacgacgcc ggccgacatc cgccgtgcca ccgaggcgga
184
                                                                                5820
          catgooggeg gtotgcacca togtcaacca ctacategag acaagcacgg tcaacttoog
185
          taccgagccg caggaaccgc aggagtggac ggacgacctc gtccgtctgc gggagcgcta
                                                                                5880
186
                                                                                5940
          tecetggete gtegeegagg tggaeggega ggtegeegge ategeetaeg egggeeeetg
187
                                                                                6000
          gaaggcacgc aacgcctacg actggacggc cgagtcgacc gtgtacgtct ccccccgcca
188
                                                                                6060
          ccagcggacg ggactgggct ccacgctcta cacccacctg ctgaagtccc tggaggcaca
189
          gggcttcaag agcgtggtcg ctgtcatcgg gctgcccaac gacccgagcg tgcgcatgca
                                                                                6120
190
          cgaggcgctc ggatatgccc cccgcggcat gctgcgggcg gccggcttca agcacgggaa
                                                                                6180
191
          ctggcatgac gtgggtttct ggcagctgga cttcagcctg ccggtaccgc cccgtccggt
                                                                                6240
192
                                                                                6300
          cctgcccgtc accgagatct gagatcacgc gttctaggat cccccgatga gctaagctag
193
          ctatatcatc aatttatgta ttacacataa tatcgcactc agtctttcat ctacggcaat
                                                                                6360
194
          gtaccagctg atataatcag ttattgaaat atttctgaat ttaaacttgc atcaataaat
                                                                                6420
195
          ttatgttttt gcttggacta taatacctga cttgttattt tatcaataaa tatttaaact
                                                                                6480
196
                                                                                6540
          atatttcttt caagatggga attaacatct acaaattgcc ttttcttatc gaccatgtac
197
          gtatcgcg
                                                                                6548
199 <210> SEQ ID NO: 5
```

Input Set : N:\Crf3\07302001\I509945.raw
Output Set: N:\CRF3\09072001\I509945.raw

200 <211> LENGTH: 6539 201 <212> TYPE: DNA 202 <213> ORGANISM: Escherichia coli LE392 203 <220> FEATURE: 204 <221> NAME/KEY: misc_feature 205 <223> OTHER INFORMATION: Clone: pTS431 206 <400> SEQUENCE: 5 aattcaagct tgacgtcagg tggcactttt cggggaaatg tgcgcggaac ccctatttgt ttatttttct aaatacattc aaatatgtat ccgctcatga gacaataacc ctgataaatg cttcaataat attgaaaaag gaagagtatg agtattcaac atttccgtgt cgcccttatt cccttttttg cggcattttg ccttcctgtt tttgctcacc cagaaacgct ggtgaaagta aaagatgctg aagatcagtt gggtgcacga gtgggttaca tcgaactgga tctcaacagc ggtaagatcc ttgagagttt tcgccccgaa gaacgttttc caatgatgag cacttttaaa gttctgctat gtggcgcggt attatcccgt attgacgccg ggcaagagca actcggtcgc cgcatacact attctcagaa tgacttggtt gagtactcac cagtcacaga aaagcatctt acggatggca tgacagtaag agaattatgc agtgctgcca taaccatgag tgataacact gcggccaact tacttctgac aacgatcgga ggaccgaagg agctaaccgc ttttttgcac aacatggggg atcatgtaac tcgccttgat cgttgggaac cggagctgaa tgaagccata ccaaacgacg agcgtgacac cacgatgcct gtagcaatgg caacaacgtt gcgcaaacta ttaactggcg aactacttac tctagcttcc cggcaacaat taatagactg gatggaggcg gataaagttg caggaccact tetgegeteg gecetteegg etggetggtt tattgetgat aaatctggag ccggtgagcg tgggtctcgc ggtatcattg cagcactggg gccagatggt aagccctccc gtatcgtagt tatctacacg acggggagtc aggcaactat ggatgaacga aatagacaga tegetgagat aggtgeetea etgattaage attggtaaet gteagaceaa gtgaagatcc tttttggctc gagtctcatg accaaaatcc cttaacgtga gttttcgttc cactgagegt cagaccecgt agaaaagate aaaggatett ettgagatee ttttttetg cgcgtaatct gctgcttgca aacaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc tegetetget aatectgtta ecagtggetg etgecagtgg egataagteg tgtcttaccg ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acggggggtt cgtgcacaca gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg agcattgaga aagcgccacg cttcccgaag ggagaaaggc ggacaggtat ccggtaagcg gcagggtcgg aacaggagag cgcacgaggg agcttccagg gggaaacgcc tggtatcttt atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag gggggcggag cctatggaaa aacgccagca acgcggcctt tttacggttc ctggcctttt gctggccttt tgctcacatg ttctttcctg cgttatcccc tgattctgtg gataaccgta ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt cagtgagcga ggaagcggaa gagcgcccaa tacgcaaacc gcctctcccc gegegttgge etgateagaa ttetteeega tetagtaaca tagatgacae egegegegat aatttatect agtttgegeg ctatattttg ttttetateg egtattaaat gtataattge gggactctaa tcataaaaac ccatctcata aataacgtca tgcattacat gttaattatt acatgcttaa cgtaattcaa cagaaattat atgataatca tcgcaagacc ggcaacagga ttcaatctta agaaacttta ttgccaaatg tttgaacgat ctgcttcgga tcctctagat tactgatttt tgtaaaggtc tgataatggt ccgttgtttt gtaaatcagc cagtcgcttg agtaaagaat ccggtctgaa tttctgaagc ctgatgtata gttaatatcc gcttcacgcc atgttcgtcc gcttttgccc gggagtttgc cttccctgtt tgagaagatg tctccgccga tgcttttccc cggagcgacg tctgcaaggt tcccttttga tgccacccag ccgagggctt

VERIFICATION SUMMARY

DATE: 09/07/2001

PATENT APPLICATION: US/09/509,945

TIME: 10:31:26

Input Set : N:\Crf3\07302001\I509945.raw Output Set: N:\CRF3\09072001\I509945.raw

 $L:5 \ M:271 \ C:$ Current Filing Date differs, Replaced Current Filing Date

STATISTICS SUMMARY DATE: 09/07/2001 PATENT APPLICATION: US/09/509,945 TIME: 10:31:26

Input Set : N:\Crf3\07302001\I509945.raw
Output Set: N:\CRF3\09072001\I509945.raw

Application Serial Number: US/09/509,945

Alpha or Numeric: Numeric

Application Class:

Application File Date: 04-04-2000

Art Unit: OIPE

Software Application: PatentIn Total Number of Sequences: 7 Total Nucleotides: 13847 Total Amino Acids: 111 Number of Errors: 0 Number of Warnings: 0 Number of Corrections: 1

MESSAGE SUMMARY

271 C: 1 (Current Filing Date differs)